THE RELATION BETWEEN THE FIXED AND FREE SALTS OF BACTERIA *

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In their study on the conductivity of bacterial cells, Green, Minneapolis and Larson [1] have shown that, at death, there is a rapid exosmosis of salts from the bacterial bodies when suspended in water. Their work suggests that some of the salts at least are free within the cell of the organism.

The present study was inaugurated for the purpose of determining the nature and relation between the fixed and free salts of bacteria, and in particular with B. coli. In this paper the salts which diffuse out of the killed bacteria are referred to as the free salts, while those which do not diffuse out but are found in the ash of bacteria which have been dialyzed, are called the fixed salts.

The bacterial cell, although minute, may nevertheless be a complex and well organized system. We cannot determine any particular specific structures in the cell, but we know that it is composed of solid particles of protoplasm suspended in a liquid medium.

This liquid, besides keeping the cell turgid against the pressure of the surrounding medium, serves as a carrier for the particles of food from the inside of the cell wall to the cell substance, and also as a carrier for the waste products of the cell back to the cell wall to pass from there into the surrounding medium. No regular circulatory system has ever been demonstrated in bacteria, but there must be some means of carrying the ions of salts, as well as other particles in solution, which have passed through the cell wall, to those parts of the cell where they are built up into new protoplasm. Whether or not the liquid passes through the cell in a regular path, carrying with it particles of food or carrying away waste materials, or whether the substances in solution simply move about in the liquid as a dispersion medium, from which they are adsorbed to the cell structures, has never been determined.

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[1] Jour. Infect. Dis., 1922, 30, p. 550.

It has frequently been pointed out that the membrane of bacteria may be lipoidal in nature. Lipoids, being surface tension depressants, would have a tendency to gather in the surface layer. Whatever this membrane is, it seems to function as an osmotic one. Particles pass into the cell wall, due to the pressure of the solution on the outside, and are released from the inner cell wall by the reduced pressure of the liquid inside of the cell. Whether the cell membrane shows any selective action to particles passing through it, or whether the whole process is simply a physical one of adsorption and osmosis, regardless of the specific nature of the particles, is still a matter of doubt. Some evidence for the former theory has been presented by Cramer [2] when he pointed out that organisms show a selective action toward phosphate as against chlorid. Again, the fact that a certain amount of chlorid is taken up by the cell and apparently, as will be pointed out later, is not utilized in building up the cell substance, is evidence of little or no selective action on the part of the cell membrane. Chlorid may, however, have a purely physiologic function, which is not yet clearly understood.

There are, then, two distinct groups of salts present in the living bacterial cell. The structural salts are those which are tied up chemically or form a part of the protoplasm of the cell. It would seem, if there is any definite or constant composition of this cell protoplasm, that the nature and amount of the structural salts should remain relatively constant, at least for the same organism, regardless of the conditions of growth. The unbound salts, however, performing, as we think of them, purely physical functions as equalizing the osmotic pressure, and preserving the turgor of the cell, must vary in amount and to a certain extent in composition with the concentration and composition of salts in the medium. An increase of the total concentration of salts in the medium would bring about an increase of salts in the cell liquid. An increase of either sodium, potassium, chloride, or phosphate in the medium would cause an increase of the corresponding substance in the cell contents. The osmotic pressure, for example, of sodium on one side of the membrane would be equalized by an equal pressure of sodium on the other side. Whether this equality of pressure for each element is maintained is not known. We know from data of former investigators [2] that cells do pile up concentrations of certain elements above those of the same element in the medium. Especially is this the

[2] Arch. f. Hyg., 1897, 28, p. 1.

case when a particular element is essential to the life process or structure of the organism and is present in comparatively low concentration in the medium. All that this may mean, however, is that the cell structure requires relatively constant amounts of certain essential elements and extracts these from the medium, no matter how low the concentration may be. Such elements then being bound up in the cell structure have little or no effect on the osmotic pressure. The excess concentration of certain salts in the cell over that in the medium may be entirely in the fixed salts, and therefore may not conflict with our theory of osmotic pressure balance.

A plausible conclusion to draw from all this is, first, that the fixed salts remain constant under different conditions of growth, at least with the same organism, and second, that the wide variations found by Cramer and others in the ash content of an organism grown on mediums of different salt concentrations are variations in the free salts only.

All of this work on diffusible salts in cells is comparatively new, and as yet there are no available data to prove or disprove the statement just made. It remains, therefore, for the present a plausible conclusion and nothing more.

In most animals and plants certain cells or groups of cells have purely structural, others purely physiologic, functions. In bacteria in a very general way certain elements compose the cell structure, while others take part in metabolism. The salts combined in the former are the structural, those connected with the latter the physiologic salts

Another question still to be answered about the diffusible salts is the one concerning the manner in which these salts are held by the cell during life. They may be adsorbed to the cell wall prior to passing into or out of the cells; they may be adsorbed to the cell substance within before actual chemical combination; or they may be in solution in the cell liquid. The broadest assumption and the most logical one would be that they occupy not one, but all three of these places, and fulfil all three functions.

Previous investigators, giving no heed to the fact that cells give off salts when killed, may have lost at least a part of these loosely held salts in the preparation of their samples for analysis through a series of washings and extractions. DeSchweinitz and Dorset [3] state as much in their paper on the ash analysis of the tubercle bacilli. It is significant for our purpose that these two investigators found no chloride in their

[3] Centralbl. f. Bakteriol., I, O., 1902, 33, p. 993.

samples, which in the present analysis does not appear in the ash but in the diffusate. It is easily seen that a part of the salts, and with that the chlorides, might have been lost by numerous washings with hot water. Also the sulphate, absent in the analyses of DeSchweinitz and Dorset, was found in this work mainly in the free salts.

The part of the mineral matter of bacteria which we here call the fixed salts may be held in the cell in several ways. They may be contained in the cell as simple insoluble inorganic salts or they may be tied to the protein molecule by adsorption or in the form of protein salts. The latter may be true especially of the heavy metal ions.

Previous to this work a rapid qualitative analysis had been made of the ash of bacterium coli. Ca, Mg, Na, K, Fe, Cl, SO_4 and P_2O_5 were found present. The method of procedure in carrying out the work of this paper is as follows: The organisms, a strain of bacterium coli, were grown on ordinary meat extract peptone broth containing 0.5% NaCl. After inoculation the flasks were incubated for 48 hours at 37 C. The organisms were then separated from the medium by centrifuging, and weighed. They were washed in distilled water to remove the broth still adhering to them and again centrifuged from the wash water. After suspending the cells again in about 6 liters of distilled water, they were killed by heat to 60 C. for 30 minutes. Immediately after heating the suspension was cooled, diluted with distilled water and allowed to stand for several hours in order to allow the salts to diffuse out. Again the liquid was separated from the organisms by centrifuging. The diffusate was evaporated to dryness and ignited to burn off the small amount of organic matter present. The organisms left after diffusion were dried and weighed and then reduced to ash in platinum. Both the ash and diffusate were analyzed quantitatively. Cl, Fe, Ca, P_2O_5, Mg, SO_4, Na and K were determined.

Green and Larson, in their conductivity experiments on bacterial suspensions, found that the electrical conductivity of the suspension remained constant for about 2 hours. After that the resistance suddenly dropped, showing that the salts do not begin to come out of the live cells until they have been suspended in distilled water for about 2 hours.

DATA FROM ANALYSIS

Total weight of moist organisms	114	gm.
Weight of dry matter in organisms	12.9260	gm.
Ash + trace of carbon	0.8392	gm.
Ash	0.7050	gm.
Amounts of different elements present in the ash		
Cl	0.0000	gm.

$Ca_3(PO_4)_2$		0.2440 gm.
	Ca	0.0936 gm.
	PO_4	0.1504 gm.
$Mg_2P_2O_7$		0.1935 gm.
	Mg	0.0417 gm.
Ba SO_4		0.0302 gm.
	SO_4	0.0125 gm.
	Fe_2O_3	0.0236 gm.
NaCl + K Cl		0.2157 gm.
K Cl O_4		0.3175 gm.
	K	0.0913 gm.
	Na	0.0184 gm.
$Mg_2P_2O_7$		0.2642 gm.
	P O_4	0.2251 gm.
Weight of diflusate dried		3.5318 gm.
Free salts + carbon		0.9425 gm.

Amounts of different elements in the free salts

AG Cl		0.2857 gm.
	Cl	0.0700 gm.
$Ca_3(PO_4)_2$		0.2223 gm.
	Ca	0.0864 gm.
	PO_4	0.1359 gm.
$Mg_2P_2O_7$		0.0882 gm.
	Mg	0.0192 gm.
Ba SO_4		0.1162 gm.
	SO_4	0.0410 gm.
	Fe_2O_3	Trace
NaCl + K Cl		0.6470 gm.
K Cl O_4		0.3375 gm.
	K	0.0936 gm.
	Na	0.1863 gm.
$Mg_2P_2O_7$		0.2944 gm.
	PO_4	0.2520 gm.
Carbon	about	0.01 gm.

DATA IN PER CENT.

Total dry matter in organisms	11.34%
Total ash in the dry matter	12.75%
Fixed salts in the ash	42.79%
Free salts in the ash	57.21%

Percentage of elements calculated on the dry weight of the ash.

Cl	0.00%
$Ca_3(PO_4)_2$ (Ca O 13.77%, P_2O_5 21.84%)	35.61%
MgO	5.92%
SO_4	1.78%
Fe_2O_3	3.35%
K	12.95%
Na	2.61%
P_2O_5	33.99%
Total	96.21%

Percentage of elements calculated on the dry weight of the free salts.

Cl	7.40%
$Ca_3(PO_4)_2$ (CaO 9.13%, P_2O_5 14.46%)	23.59%
Mg O	2.04%
S O_4	4.36%
Fe_2O_3	Trace
K	9.94%
Na	19.77%
P_2O_5	26.84%
Carbon	1 +
Total	96.94%

An analysis was made also of the mineral constituents of the broth used for the cultivation of the organisms. The results of this analysis are as follows:

Percentage of ash in the broth 0.9%

Percentage of constituents of the ash in broth.

NaCl	0.5%
P_2O_5	0.2%
Ca + Mg	0.1%

Small amounts and traces of K, SO_4 and Fe.

In looking over the results of this work, we find that the contents of dry matter and total ash for bacterium coli come within the range found by other investigators for other organisms. The water content seems to be relatively constant for all organisms analyzed up to the present time.

A significant factor for our purpose is that the weight of the free salts is greater than that of the fixed salts. This shows that not only do salts come out of bacterial cells when the latter are killed by heat and suspended in distilled water, but the greater part, 57.21% of the total salts in the cells, diffuse out on the death of the organisms. The total absence of chloride in the fixed salts and its presence in the unbound salts shows, according to our theory, that chloride is not essential in the cell structure but is used to equalize the pressure of chloride in the medium or in some other unknown function. Sulphates are present to a much greater extent in the diffusate than in the ash. Iron is present almost entirely in the fixed salts. The function of iron in the cell is not known. If it serves, as in the cells of higher animals, as an oxygen carrier, its function is a biologic one. Phosphate is by far the most abundant element. It seems to be the essential element in the cell structure, as well as in other functions of the cell, and is often found in higher concentrations in the organisms than in the medium.

Sodium and potassium show interesting results. Both are found in the free salts, but sodium predominates. This was to be expected. With a concentration of 0.5% NaCl in the broth the organisms would have to take up a considerable quantity of sodium to equalize the osmotic pressure caused by the sodium ions in the broth. The fact that this element appears almost entirely in the diffusible salts suggests that its action in the cell is probably physiologic or physical. The results for sodium and potassium obtained here agree very well with analyses of cells of higher plants and animals, in which potassium but no sodium is found present. Potassium is invariably the constituent of cell substance while sodium is present in the fluids that have purely physiologic functions in the bodies of animals and in plants. In red blood cells, for instance, potassium is always present, but sodium has never been found. The large amount of sodium found by Cramer and others in bacteria must be almost entirely in the unbound salts. It is not improbable that the small amount of sodium in the fixed salts could be replaced by potassium by a little higher concentration of that element in the medium. The amount of potassium present in the

diffusate is almost four times as large as the amount of sodium in the ash. This shows that potassium is utilized by the cell in its structure and also has a biologic function.

SUMMARY

It has been shown again that salts diffuse out of bacterial cells when they are killed by heat and suspended in distilled water. Moreover, it has been shown that the amount of free salts in bacteria is greater than the amount of fixed salts. The two groups of salts contain about the same constituents in somewhat different proportions. Chloride and iron are the exceptions, occurring only in the diffusate and in the ash, respectively. The results for sodium and potassium agree well with those found, in the past, in analyses for the same elements in higher plant and animal cells.

The results obtained in this investigation may be typical of other organisms cultivated under similar or different conditions. This fact would, however, have to be determined by added experiments. The results can be regarded as established only for B. coli cultivated and treated as described in this paper.